

Attorney Docket No.: T5530.CIP (UT-0006)
Inventors: Rao et al.
Serial No.: 09/109,858
Filing Date: July 2, 1998
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This listing of the claims will replace all prior versions and listings of claims in the application:

Listing of the claims:

Claims 1-11 (canceled)

Claim 12 (previously amended): A method of isolating a pure population of rodent or human CNS neuron-restricted precursor cells comprising the steps of:

(a) isolating a population of rodent or human multipotent CNS stem cells which generate both neurons and glia;
(b) incubating the multipotent CNS stem cells in NEP medium;

(c) replating the multipotent CNS stem cells on laminin in NEP medium in the absence of chick embryo extract to induce cell differentiation;

(d) removing A2B5+ cells from the differentiating cells via specific antibody capture with an antibody that specifically recognizes A2B5;

(e) purifying from the supernatant following step (d) a subpopulation of cells expressing embryonic neural cell adhesion molecules via a procedure selected from the group

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consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture, wherein said procedure uses an embryonic neural cell adhesion molecule antibody that specifically recognizes polysialated neural cell adhesion molecule (NCAM); and

(f) incubating the purified subpopulation of cells in a FGF-containing medium to obtain an isolated, purified population of rodent or human CNS neuron-restricted precursor cells.

Claims 13-14 (canceled)

Claim 15 (previously amended): The method of claim 12 wherein the subpopulation of cells expressing embryonic neural cell adhesion molecules is purified by specific antibody capture.

Claim 16 (previously amended): The method of claim 12 wherein the rodent or human multipotent CNS stem cells are neuroepithelial cells.

Claims 17-20 (canceled)

Claim 21 (previously amended): A method of isolating a pure population of rodent or human CNS neuron-restricted precursor

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cells comprising the steps of:

(a) removing a sample of spinal cord tissue from a rodent or human embryo at a stage of embryonic development after closure of the neural tube;

(b) dissociating cells comprising the sample of spinal cord tissue removed from the embryo;

(c) removing A2B5+ cells from the dissociated cells via specific antibody capture with an antibody that specifically recognizes A2B5;

(d) purifying from the supernatant following step (c) a subpopulation expressing embryonic neural cell adhesion molecule via a procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture, using an embryonic neural cell adhesion molecule antibody that specifically recognizes polysialated neural cell adhesion molecule;

(e) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a FGF-containing medium; and

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(f) incubating the plated cells in the FGF-containing medium to obtain an isolated, pure population of neuron-restricted precursor cells.

Claims 22-23 (canceled)

Claim 24 (previously amended): The method of claim 21 wherein the subpopulation of cells expressing embryonic neural cell adhesion molecules is purified by is specific antibody capture.

Claim 25 (canceled)

Claim 26 (previously amended): A pure population of rodent or human CNS neuron-restricted precursor cells isolated by the method of claim 12.

Claim 27 (previously amended): A pure population of rodent or human CNS neuron-restricted precursor cells isolated by the method of claim 21.

Claims 28-60 (canceled)